# Cockroach allergens: Coping with challenging complexity

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The report by Glesner et al[1] studied the relative potency of 12 nonstandardized German cockroach extracts and 8 allergenic molecules (Bla g 1, 2, 4, 5, 6, 9, and 11 and Per a 7) by using direct human IgE antibody binding with ImmunoCAP assays and in-house competitive IgE antibody inhibition assays. Importantly, the investigators included 3 new allergens (Bla g 6, 9, and 11) and used a robust study design involving individual sera, various extracts, and a comprehensive list of allergens.

Several important issues are raised by the study. First, allergen extracts of German cockroach (*Blattella germanica*; an overview of arthropod single allergens is provided in Hilger et al[2]) with potential use in diagnostics and allergen immunotherapy (AIT) are highly variable in their individual allergenic protein content. Cockroach allergen composition and concentration both differed, presumably depending on whether fecal pellets and body parts of juvenile versus adult insects were extracted. This study elegantly assesses the extract composition by detecting the major individual allergens Bla g 1, Bla g 2, and Bla g 5 (Fig 1 and Table I).[1] The 12 extracts showed different single cockroach allergen concentrations and widely dissimilar potencies by using competitive IgE antibody inhibition assays with 5 individual sera.

Second, IgE anti-cockroach binding to the 8 cockroach allergens displayed significant differences between 23 subjects. Some subjects had no IgE to the panel of 7 tested *Blattella germanica (Bla g)* allergens and Per a 7 (98% surrogate for Bla g 7), presumably because of IgE reactivity to unidentified *B germanica* allergens. Other subjects showed variable IgE levels to an increasing number of cockroach allergens, which correlated with specific IgE binding to whole *Bla g* extract (ImmunoCAP singleplex; Phadia, Thermo Fisher Scientific, Uppsala, Sweden). Thus the allergen panel included most of the important *Bla g* allergens relevant to the study population, but it missed some allergens.

Importantly, this detailed work clearly identifies 2 fundamental reasons for heterogeneity, namely (1) the individual allergen distribution in the *Bla g* sources and extracts and (2) the heterogeneous *Bla g*–specific IgE repertoires in individual subjects. These points should be considered by the allergist when attempting a proper diagnosis and considering the degree of cockroach allergen standardization.

Cockroach adult bodies, nymphs, egg sacks, and feces contribute differentially to patient sensitization and levels of individual allergens in the final extract. Feces contain high concentrations of Bla g 1 and Bla g 2 but no Bla g 5 compared with whole-body extracts. Extraction methods also greatly influence the presence or absence of particular allergens in the final extract. Similar findings have been demonstrated with extracts of other arthropod allergen sources, including house dust mites. Therefore allergen manufacturers had to extract allergens from house dust mite bodies and feces separately to control the relative levels of the important (major) allergens in a final "recombined" preparation in mite tablets used for immunotherapy.[3] Perhaps separate extractions of bodies and feces of cockroaches represent a potential solution, but the availability of a set of individual allergen immunoassays that could define potency would help because only 3 important *B germanica* allergens are being monitored at present.

In the United States cockroach allergen preparations are still considered nonstandardized extracts. Since 2001, the US Food and Drug Administration has worked on enhanced characterization and standardization of cockroach allergens. Despite multiple *Bla g* allergens being identified and characterized, a correlation of their presence to the overall biological activity of cockroach extracts has not yet been demonstrated. In addition, biological methods using skin test titration (eg, $ID_{50}EAL$) have not correlated with direct measurements of Bla g 1, Bla g 2, or Bla g 5 levels.[4] From the present data,[1] these results should not be surprising.[5] Recently, the US Food and Drug Administration has attempted to establish a multiplex allergen extract potency assay[6] for estimation of overall potency and specific composition assessment. Another alternative approach has involved use of mass spectrometry to successfully quantify Bla g 1 to Bla g 5.[7] However, neither approach covers all important cockroach allergens.

Heterogeneous *Bla g*–specific IgE antibody repertoires among allergic patients contribute to the complexity of producing a highly predictive test for a clear determination of immunodominant *Bla g* allergens. The extremely variable pattern of IgE antibody responses to various *Bla g* allergens is shown in Fig 2 of Glesner et al.[1] In contrast, dust mites appear to express only 3 major allergen (groups 1, 2, and 23).[8] For German cockroach, it has been difficult to confirm the true major allergens (eg, Bla g 2 and Bla g 11) based solely on IgE antibody binding to limited populations of subjects (Fig 3 in Glesner et al[1]). Interestingly, other allergen sources, such as birch pollen, ragweed pollen, cat dander, or *Alternaria alternata*, display fewer dominating major allergens

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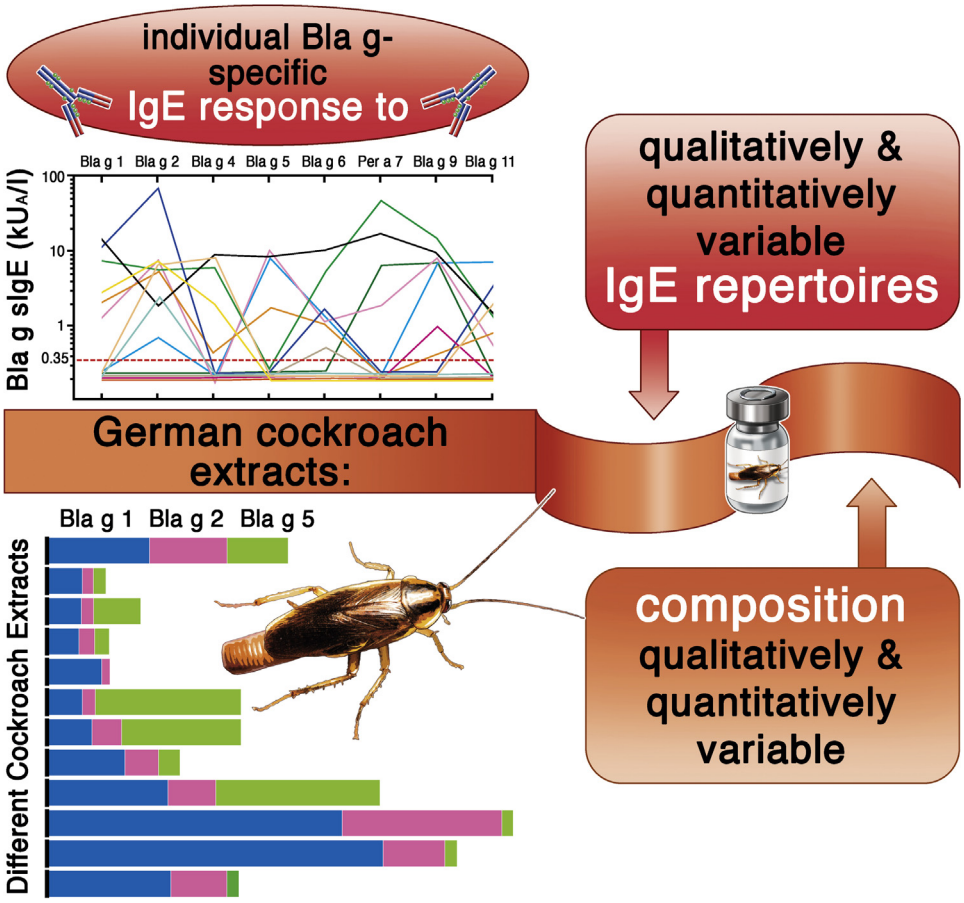


**FIG 1.** German cockroach allergen extracts display variable potencies (represented by a *wavy line*). Heterogeneous IgE repertoires (*upper left panel*, with individual allergen-specific IgE levels to single cockroach allergens) and complex allergen composition *(lower left panel)* contribute to the complexity.

**TABLE I.** German cockroach *(Blattella germanica, Bla g)* allergens (http://allergen.org/)

| Allergen | Name/type/function | Molecular weight (kDA) |
|---|---|---|
| Bla g 1 | Nitrile specifier protein | 46 |
| Bla g 2 | Aspartic protease | 36 |
| Bla g 3 | Hemocyanin | 79 |
| Bla g 4 | Calycin | 21 |
| Bla g 5 | Glutathione-S-transferase | 23 |
| Bla g 6 | Troponin C | 21 |
| Bla g 7/Per a 7 | Tropomyosin | 31 |
| Bla g 8 | Myosin, light chain | — |
| Bla g 9 | Arginine kinase | 40 |
| Bla g 11 | α-Amylase | 57 |

(Bet v 1, Amb a 1, Fel d 1, and Alt a 1), whereas cockroaches seem to induce exceptionally diverse IgE sensitization patterns, more than other complex allergen sources, such as grass pollen[9] or house dust mites.[8] Ultimately, the authors question the value of establishing potency units for cockroach based on a single test that ignores the broad spectrum of allergens recognized by different subjects. A single standardized extract would need to have all the major allergens for use in diagnosis and immunotherapy.

Preparation of cockroach extracts should involve use of different allergen sources and appropriate extraction methods to maximize the breadth of known cockroach allergens. Allergens listed by the World Health Organization/International Union of Immunological Societies Allergen Nomenclature Sub-Committee Allergen Database (http://allergen.org/)[10] provide good diversity, but new published studies are needed to insure a comprehensive allergen profile.

Potential allergen standards by competent regulatory authorities should consider not only variation in extract composition and potency of sources but also the heterogeneity of IgE-antibody repertoires recognized by allergic subjects.

The allergist should be aware of different performance characteristics of commercially available cockroach extracts from different companies and those designed for skin prick tests or allergen-specific IgE testing[5] and the potentially variable results caused by absent, low-level, or as yet unidentified *B germanica* allergens. The performance characteristics and value of component-resolved diagnostics should also be considered.

AIT studies should use the most complete *B germanica* extracts available that have been "standardized" by using a variety of qualitative and quantitative *in vitro* and *in vivo* assay methods. Importantly, the allergen manufacturer should list the content of major

*B germanica* allergens in the extracts. Ideally, composition of single allergens in AIT extracts should reflect the individual patient's IgE repertoire rather than the "one size fits all" approach. However, at present, a truly patient-tailored AIT is not possible. Therefore balanced allergen preparations for cockroach AIT will remain a compromise. For the future, the challenge involves the question of how to properly standardize cockroach allergen extracts. From a European viewpoint, appropriate dose-finding studies would also be necessary to establish an optimal AIT dose that effectively balances the efficacy and safety of the final product.

In conclusion, candidate diagnostic and AIT cockroach extracts for studies, such as the Inner-City Asthma Consortium cockroach immunotherapy trial (NCT02513264), but also for routine diagnostic and treatment use need to be verified and documented to ensure they contain appropriate allergens and concentrations of the major sensitizing molecules for the study population. This will require initial verification of the IgE antibody heterogeneity within the study population and subsequent selection and verification of a candidate cockroach extract to confirm its comprehensive nature by using direct and antigen inhibition immunoassays and possibly mass spectrometry.

## REFERENCES

1. Glesner J, Filep S, Vailes LD, Wunschmann S, Chapman MD, Birrueta G, et al. Allergen content in German cockroach extracts and sensitization profiles to a new expanded set of cockroach allergens determine in vitro extract potency for IgE reactivity. J Allergy Clin Immunol 2019;143:1474-81.e8.
2. Hilger C, Kuehn A, Raulf M, Jakob T. Cockroach, tick, storage mite and other arthropod allergies: where do we stand with molecular allergy diagnostics? Part 15 of the Series Molecular Allergology. Allergo J Int 2014; 23:172-8.
3. Henmar H, Frisenette SM, Grosch K, Nielsen K, Smith G, Sonderkaer S, et al. Fractionation of source materials leads to a high reproducibility of the SQ house dust mite SLIT-tablets. Int Arch Allergy Immunol 2016; 169:23-32.
4. Slater JE, James R, Pongracic JA, Liu AH, Sarpong S, Sampson HA, et al. Biological potency of German cockroach allergen extracts determined in an inner city population. Clin Exp Allergy 2007;37:1033-9.
5. Kleine-Tebbe J, Linneberg A, Johansen N, Larsen JN, Nolte H, Ipsen H. Skin prick test (SPT) and specific IgE may measure different IgE reactivities and be complementary in allergy diagnosis. Allergy 2018;73(suppl 105):39.
6. deVore NC, Huynh S, Dobrovolskaia EN, Slater JE. Multiplex microbead measurements for the characterization of cat and ragweed allergen extracts. Ann Allergy Asthma Immunol 2010;105:351-8.
7. Mindaye ST, Spiric J, David NA, Rabin RL, Slater JE. Accurate quantification of 5 German cockroach (GCr) allergens in complex extracts using multiple reaction monitoring mass spectrometry (MRM MS). Clin Exp Allergy 2017;47:1661-70.
8. Thomas WR. IgE and T-cell responses to house dust mite allergen components. Mol Immunol 2018;100:120-5.
9. Tripodi S, Frediani T, Lucarelli S, Macri F, Pingitore G, Di Rienzo Businco A, et al. Molecular profiles of IgE to Phleum pratense in children with grass pollen allergy: implications for specific immunotherapy. J Allergy Clin Immunol 2012; 129:834-9.e8.
10. Pomes A, Davies JM, Gadermaier G, Hilger C, Holzhauser T, Lidholm J, et al. WHO/IUIS allergen nomenclature: providing a common language. Mol Immunol 2018;100:3-13.